Pre- Ph.D. Registration Synopsis

Bioethanol Production and its Optimization Utilizing *Shorea robusta* C.F.Gaertn. Seed Cake

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STATE OF THE ART

Biofuels are gaining an impetus to address the rampant issue of energy demand in an eco-friendly and cost-effective manner. The proposed work is an endeavour to bring the transformations, by involving biological components. It envisages the application of starch degrading enzymes and yeast for biofuel such as, bioethanol generation from underutilized seed biomass. Ethanol, also termed as ethyl alcohol, grain alcohol, or pure alcohol, is often abbreviated as EtOH. It is a light alcohol and is a colourless, volatile, flammable liquid with a characteristic odour and burns with an almost invisible flame[1].

Bioethanol is primarily used to blend with petrol or can be used directly in vehicles, thereby minimizing greenhouse gas emissions and consumption of petrol. Apart from its major application in blending, it can be employed for power generation by fuel cells and thermal combustion. In addition, it can also be utilized as feedstock in chemical industry for various product generation[2].

Shorea robusta C.F.Gaertn. commonly recognized as Sal, belongs to the Dipterocarpaceae family. Sal seed is an important non-timber forest product (NTFP) found in various South Asian countries like India, Nepal, Pakistan, Bangladesh, Myanmar and Bhutan. Recommendation from the Planning Commission of India for Sal seed has opened up potential for advancement as NTFP. Annually, the Sal seed availability has been estimated to be 1.5 million tons, with the dependence of 2030 million forest dwellers on Sal seed, leaves, and resin collection[3]. Jharkhand has an immense scope of collecting minor forest produce as more than 30% of the land is under forest cover and produces nearly 1,00,000 MT of Sal seeds annually, indicating its high abundance[4].

The significant component of Sal seed is oil, with 34.6 % having moisture and ash content of 8.46 % and 6 %, respectively[5]. Owing to its high oil content, it is primarily used for oil extraction, which is significant in the production of triglycerides. The de-oiled cake (after oil extraction) contains nearly 40-50 % starch[6] and is used for boiling plants and as cattle feed after standardization[7]. Till date, only the fat portion of the Sal seed has been used for consumption and soap production. The carbohydrate content, mainly starch, of the Sal seed is not exploited much for biofuel generation.

Most of the work on bioethanol production has been done utilizing lignocellulosic biomass. In a study, 7.62 % (v/v) ethanol was achieved through a mixed enzymatic venture utilizing Kans

grass as a lignocellulosic biomass[8]. In another work, 71.39 mg/mL of ethanol was reported after 72 h of fermentation utilizing wheat bran and coffee pulp as lignocellulosic substrates[9].

The scientific literature on Sal seed and Sal seed cake exploitation for bioethanol production is scarce and superficial. Bioethanol production (10 %) was reported from Sal seeds after 72 h of fermentation. The same study examined the effect of different parameters, namely temperature, incubation time, pH, and nutrients, for bioethanol production without optimization[10]. Similarly, Sal saw dust was employed as a substrate where 9.4 g/L of bioethanol was reported in an optimized condition after 24 h of incubation[11].

Recently, oilseed cakes have gained attention because of their high protein and carbohydrate content. The seed cakes serve as a potential feedstock for value-added product generation such as biofuel, biopolymer, biogas, enzymes, etc. Moreover, tapping oilseed cakes as a substrate would lessen environmental pollution and production costs[12]. Few literature reports are available on bioethanol production from oil seed cakes wherein about 6.01 g/L ethanol was reported from pressed cakes of Jatropha oil seeds using *Saccharomyces cerevisiae as a* fermentative organism[13]. In another study, 5.57 ml/L of ethanol was produced from Jatropha oil seed cake using yeast strains *Candida tropicalis* (Y-26) and *Saccharomyces cerevisiae*[14]. In addition, 88.62 g ethanol kg^{-1} dry seed residue was reported utilizing de-oiled *Pongamia pinnata* seed cake as a substrate and *Saccharomyces cerevisiae* as fermenting microorganism[15].

There are no reported studies on bioethanol generation from Sal seed cake. Thus, an attempt is made to utilize the Sal seed cake for bioethanol production.

PROBLEM TO BE INVESTIGATED

Lignocellulosic biomass mainly contains cellulose (40–60 %), hemicellulose (20–30 %), and lignin (15–30 %)[16]. Lignin is a complex aromatic polymer containing phenylpropanoid subunits linked by C-C and C-O bonds. It is closely associated with hemicellulose forming a cover to wrap the crystalline cellulose micro-fibrils, which impedes the accessibility of enzymes toward hydrolysis[17]. Degradation or removal of lignin is important for accessing the cellulose and hemicellulose during hydrolysis. Thus, the pretreatment method is important in bioethanol production process[18]. India mainly depends on sugarcane production and damaged food grains for ethanol production. Further, the utilization of sugarcane results in food versus

fuel issues. Moreover, only the surplus production of sugarcane and the availability of damaged food grains (rice and wheat) would facilitate sustainable bioethanol production. In this regard, the high abundance of Sal seed in Jharkhand can be a potential biomass for bioethanol production. Also, the biomass pretreatment process is not required due to the absence of lignin in Sal seed, which would further make the process cost-effective.

OBJECTIVES OF THE INVESTIGATION & MOTIVATION

Based on the above literature survey and research gap on bioethanol production from various biomass sources and to solve the above research issues the following objectives were framed:

1. **Biochemical compositional analysis of the Sal seed cake.**
2. **Simultaneous saccharification and fermentation (SSF) of Sal seed cake for bioethanol production and its optimization studies.**
3. **Comparative characterization study of raw and residual biomass.**

MOTIVATION BEHIND THE WORK

Unlike in Brazil and the USA, where bioethanol is produced mainly from sugar and corn starch, respectively, at competitive prices, first generation biofuels cannot be produced in developing countries such as India due to concerns about food security. In India, only surplus sugar and damaged grains have been used for bioethanol production, which mainly depends on yield and climatic conditions. The main motivation behind this work is to utilize the untapped forest residue for cost effective production of lignocellulosic fuels such as bioethanol that do not compete with the existing food supply.

HYPOTHESIS

Ethanol production is an age-old process. Though many studies have been reported on lignocellulosic ethanol production, the commercial viability of using lignocellulosic as the substrate has not yet been established. Thus, in the present scenario, emphasis has been given to the studies on some specific raw materials that are regionally specific, and abundantly found in Jharkhand. Owing to its easy availability and containing a higher percentage of starch, the production of ethanol from the starchy biomass can be economically viable. Majority of the

cost is contributed by the price of raw material and pretreatment process. With the identification of Sal seed as the raw material for bioethanol production, the proposed study has been formulated wherein with the minimum effort, the process can be made commercially feasible.

In this context, an attempt has been made to explore the under-utilized seed cake biomass for bioethanol production. Further, enzymatic processes will be adopted as they offer major advantages such as, minimal byproduct formation, high yields, low chemical disposal costs, and mild operating conditions over conventional processes.

RESEARCH DESIGN AND METHODOLOGY

MATERIALS REQUIRED

Substrate, enzymes, yeast, equipment and chemicals used in the present study have been described in this section.

- Sal seed cake as a substrate will be collected from local oil mills (Ratu Block).
- Fungi and yeast (*Saccharomyces cerevisiae*) will be procured from outside and maintained in the lab itself.
- Starch degrading enzymes (amylases) will be produced in lab followed by its activity analysis.
- Scanning Electron Microscopy (SEM), Bomb Calorimeter, Fourier Transform Infra-red Spectroscopy (FTIR), X-ray Diffraction (XRD), and Gas Chromatography- Mass Spectrometry (GC-MS).
- Muffle furnace, Weighing balance, Spectrophotometer, Centrifuge, Autoclave, Hot air oven, Water bath etc.

CHEMICALS TO BE USED

All the analytical grade chemicals to be used in various experimental studies are given below:

Agar, Anthrone, Ammonium chloride, Ammonium sulphate, Bovine Serum Albumin (BSA), Calcium chloride, 3,5-dinitrosalicylic acid (DNS), Ethanol, Folin-Ciocalteu Reagent (FCR), Glucose, Potassium chloride, Potassium dihydrogen phosphate, Polysorbate 80 (Tween 80), Potassium iodide, Potassium permanganate, Potassium sodium tartrate, Potassium sulphate etc.,

TOOLS & SOFTWARES

- Scanning Electron Microscopy (SEM), Bomb Calorimeter, Fourier Transform Infra-Red Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Gas Chromatography- Mass Spectrometry (GC-MS).
- Muffle furnace, Weighing balance, Spectrophotometer, Centrifuge, Autoclave, Hot air oven, Water bath etc.,
- Minitab and Origin.

METHODOLOGY

1. **Sample collection and its processing into powder biomass:**

   Sal seed cakes will be collected from local oil mills and processed into powder biomass by an electrical grinder.

2. **Biochemical compositional analysis of the seed cake powder biomass:**

   Proximate analysis (Ash, Moisture, Total solid, Volatile content) will be done according to the standard methods of Association of Official Analytical Chemists[19]. Compositional analysis will follow standard methods such as, Anthrone method[20] for starch estimation, and Dinitrosalicylic acid (DNS)[21] method for reducing sugar analysis.

3. **Amylolytic enzyme production through Solid State Fermentation:**

   Alpha amylase and Glucoamylase production will be done through solid state fermentation using a fungal strain with different substrates. After fermentation, contents will be mixed with distilled water followed by centrifugation and the collected supernatant will be used for enzyme activity analysis. Alpha amylase and Glucoamylase activity will be determined by following the standard methods[22-23].

4. **Simultaneous Saccharification and Fermentation (SSF) of the seed cake powder biomass and its optimization:**

   Simultaneous saccharification and fermentation of the powdered seed cake biomass for ethanol production will follow the standard methodology[24]. Process optimization will be performed through Response Surface Methodology (RSM). The ethanol concentration of the fermented sample will be estimated by Dichromate oxidation method[25].

5. **Structural characterization of the raw and fermented residual solid:**

   The efficacy of the production process will be determined through structural characterization of the raw and fermented residual solid which will be done through various techniques such as Scanning Electron Microscopy (SEM), Bomb Calorimeter, Fourier Transform Infra-Red Spectroscopy (FTIR), X-ray Diffraction (XRD), and Gas Chromatography- Mass Spectrometry (GC-MS) analysis for products profiling.

SIGNIFICANCE OF THE STUDY

The proposed study has the following significance:

1. Effective utilization of the underutilized Sal seeds for seed cake biomass production and its end uses.
2. It will help to develop an efficient methodology for the maximum energy production in the form of bioethanol.

IT'S RELEVANCE TO PRESENT DAY PROBLEMS AND NEED OF THE SOCIETY

1. India currently relies upon sugarcane and damaged grains for bioethanol production, which very much depends on climatic conditions. Any fluctuations or significant changes in climatic conditions lead to a low yield of sugarcane that will affect the bioethanol production process.

2. Further, technology for lignocellulosic bioethanol production is well established but not fully commercialized.
3. Diverting sugarcane for bioethanol production also leads to food vs. fuel issues.
4. Employment of starchy biomass that, too, without lignin and owing to its high availability, will circumvent the feedstock issue for bioethanol production.
5. The Sal seeds are available in surplus in the remote villages of Ranchi district of Jharkhand where most of the local peoples are mainly involved in Sal seed collection.
6. The successful operation of the process will generate employment in the local communities. Apart from the cash crop income, people belonging to the poor communities and self-help groups (SHGs) of the villages earn additional income by selling the Sal seeds (substrates for ethanol production).

CONTRIBUTION TO THE EXISTING KNOWLEDGE

1. Proximate and compositional analysis of Sal seed cake biomass will help examine its feasibility for bioethanol production.
2. Enzymatic valorization of the Sal seed cake will further contribute to an eco-friendly methodology development.
3. Characterization of the Sal seed cake will impart an in-depth knowledge about its intricate structural changes that will authenticate the efficacy of the process.

FUTURE WORK

1. The fermented residual solid biomass may be further utilized for bio manure production.
2. Doped nanoparticles may be employed for bioethanol generation instead of enzymes.
3. Further work may be initiated based on the GC-MS profiling of the fermented liquid for the identification of possible phytochemicals.

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